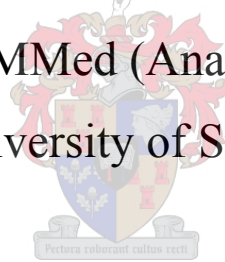


Comparison of haemoglobin values measured at point of care with the standard laboratory value

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Declaration

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Abstract

Point of care (POC) haemoglobin (Hb) concentration estimations guide acute decisions on red blood cell transfusion. We studied the accuracy of three POC devices when compared to central laboratory Hb testing and how between-method Hb disagreements could affect the decision to transfuse.

The Health Research Ethics Committee of the University of Stellenbosch approved a method comparison study of perioperative Hb concentration measurement in arterial blood sampled from 58 adult cardiothoracic surgery patients. The Hb concentration was measured by using two Ilex GEM Premier™ 3500 blood gas analysers (Blood gas A and Blood gas B), a HemoCue® Hb 201⁺ System (HemoCue), and our central laboratory's Siemens Advia® 2120 flow cytometry system (Laboratory haemoglobin). We regarded that a between-method Hb difference exceeding 10% (1 g/dL at an Hb value of 10 g/dL), would likely erroneously influence the transfusion decision. Furthermore, one unit of packed red blood cells will increase the Hb by ± 1 g/dL and clinically represents the smallest transfusion associated risk that a patient will be exposed to.

From the 58 patients included in the study, 70 central laboratory Hb concentration (Laboratory haemoglobin) measurements and 58, 72, and 71 measurements by the HemoCue, blood gas analyser A (Blood gas A) and blood gas analyser B (Blood gas B), respectively were taken.

We found that the mean POC (HemoCue, Blood gas A, and Blood gas B) Hb levels underestimated the mean central laboratory Hb level by 0.79 g/dL, 0.81 g/dL, and 0.67 g/dL, respectively. The 95% confidence interval (CI) of the between-method difference revealed that unlike the Blood gas A (0.43 to 1.15 g/dL) and Blood gas B (0.46 to 1.16 g/dL) values, the HemoCue (0.47 to 0.87 g/dL) values did not breach the predetermined 1 g/dL limit.

A Bland-Altman analysis revealed similar between-method mean Hb differences. However, the HemoCue upper and lower limits of agreement (LOA) were narrower, and the 95% CI of the LOAs do not overlap with those of Blood gas A and Blood gas B. Overall the HemoCue is more accurate.

Also, the 95% CI of the HemoCue's lower LOA was below 1 g/dL. This device does not give readings that clinically overestimate the Laboratory haemoglobin.

Similarly, the mountain plot demonstrates the greater agreement and precision of the HemoCue device, as compared to Blood gas A and Blood gas B.

An error grid analysis focused on the Hb 6 to 10 g/dL clinical decision-making range where only a 10% error was permitted. The error grid analysis revealed that the HemoCue and blood gas analysers had potential transfusion errors of less than 5% and more than 20%, respectively. However, none of the evaluated devices produced values in the major therapeutic error zone, where a decision to transfuse or not transfuse blood will be made erroneously.

Opsomming

Punt van behandeling toetsing van hemoglobien (Hb) konsentrasies rig tydige besluitneming aangaande rooibloedseltransfusies. Ons het die akuraatheid van drie punt van behandeling toestelle ondersoek en vergelyk met die sentrale laboratoriumwaardes en hoe dit die besluit om te transfuseer kon beïnvloed.

Toestemming om 'n metode vergelykende studie uit te voer is van die Gesondheidsnavorsing Etiese Komitee van die Universiteit van Stellenbosch verkry. Arteriële bloedmonsters is geneem van 58 volwasse pasiënte wat kardiotorakschirurgie ondergaan het. Hemoglobien konsentrasies is bepaal met twee Ilex GEM Premier™ 3500 bloedgas analiseerders (Blood gas A en Blood gas B), 'n HemoCue® Hb 201⁺ System (HemoCue) en die sentrale laboratorium se Siemens Advia® 2120 vloesitometrie sisteem (Laboratory haemoglobin).

'n Tussen-metode verskil van meer as 10% (1 g/dL teen 'n Hb waarde van 10 g/dL) is gebruik om 'n noemenswaardige verskil aan te dui aangesien so 'n fout die besluit om te transfuseer moontlik verkeerdelik kan beïnvloed. Ook vermeerder een eenheid gepakte rooibloedselle die Hb waarde met ongeveer 1 g/dL en verteenwoordig klinies die kleinste transfusie geassosieerde risiko waaraan 'n pasiënt blootgestel word.

Van die 58 pasiënte wat in die studie ingesluit is, is 70 laboratorium Hb meetings en 58, 72 en 71 meetings van die HemoCue, Blood gas A en Blood gas B onderskeidelik geneem.

Ons het bevind dat die punt van behandeling toestelle (HemoCue, Blood gas A en Blood gas B) die gemiddelde laboratorium Hb waardes onderskat het met onderskeidelik 0.79 g/dL, 0.81 g/dL en 0.67 g/dL. Die 95% vertrouensinterval van die tussen-metode verskil het aangedui dat die Blood gas A (0.43 tot 1.15 g/dL) en Blood gas B (0.46 tot 1.16 g/dL) die voorafbepaalde 1 g/dL limiet oorskrei. Die HemoCue, hierteenstaande, het nie die limiet oorskrei nie (0.47 tot 0.87 g/dL).

Hierdie bevindinge is bevestig met 'n Bland-Altman analise. Die boonste en onderste limiete van ooreenstemming (LOA) van die HemoCue was egter nouer as die van die Blood gas A en Blood gas B en die 95% vertrouensinterval van die LOA oorvleuel nie met die van Blood gas A en Blood gas B nie. In die geheel beskou is die HemoCue meer akkuraat.

Verder is die HemoCue se 95% vertrouensinterval vir die onderste limiet minder as 1 g/dL. Hierdie toestel se resultate het dus nie die sentrale laboratorium se Hb waarde klinies oorskat nie.

Die verspreidings diagram (mountain plot) demonstreer ook die beter ooreenstemming en akkuraatheid van die HemoCue toestel in vergelyking met die van Blood gas A en Blood gas B.

‘n ‘Error grid analysis’ het gefokus op die Hb 6 tot 10 g/dL klinies belangrike reikwydte waar slegs ‘n 10% fout toelaatbaar is. Weereens was die HemoCue meer akuraat met potensiële transfusie foute van minder as 5% teenoor die bloedgas analiseerders se meer as 20% potensiële foute. Geen waardes het egter in die major terapeutiese foutsone geval waar ‘n besluit om te transfuseer al dan nie tot skade vir die pasiënt kan lei nie.

Acknowledgement

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List of Abbreviations

ABG	Arterial blood gas
Blood gas A	Blood analysed by the first Ilex GEM Premier™ 3500 blood gas analyser
Blood gas B	Blood analysed by the second Ilex GEM Premier™ 3500 blood gas analyser
CBC	Complete blood count
CI	Confidence interval
EDTA	Ethylenediaminetetraacetic acid
Hb	Haemoglobin/hemoglobin
Hct	Haematocrit
HemoCue	HemoCue® Hb 201 ⁺ System
HiCN	Cyanmethaemoglobin assay
ICSH	International Council for Standardisation in Haematology
iQM	Intelligent quality management
LOA	Limits of agreement
NCCLS	National Committee for Clinical Laboratory Standards
POCD	Point of care device
POCT	Point of care testing
SD	Standard deviation
USA	United States of America

1. INTRODUCTION

1.1 Background

Point of care testing (POCT) is medical diagnostic testing that is performed outside the clinical laboratory, at or near the site of patient care, according to the College of American Pathologists.^{1,2,3,4,5,6,a} The driving notion behind POCT is that a result can be generated quickly and the appropriate treatment implemented faster than if the specimen was sent to a laboratory for analysis.¹ Technology has improved to the extent that POCT is employed in an ever-increasing number of settings for a variety of diagnostic purposes. Pecoraro and colleagues recently raised concerns regarding the value of POCT in a systematic survey.⁷ Firstly, the impact of POCT on clinical outcomes remains to be proven as Pecoraro et al. observed that only 13% of the 84 studies included in their systematic survey evaluated the impact of POCT on clinical practice. Secondly, they questioned whether the improvement in technology is in response to a clinical need or to marketing strategies leading to the perception that this technology is needed. Thirdly, the accuracy of POCT compared to formal laboratory investigations have to be determined, forming the focus of this investigation.

The clinical importance of POCT is easier to translate into practice in an environment where there can be rapid changes in the haemodynamic stability of patients, such as before or during surgery. In our “developing-world” setup, results from POCT, specifically arterial blood gas (ABG) analysis, are used daily to diagnose life-threatening anaemia and initiate or guide transfusion of packed red blood cells; accurate and timeous results being of utmost importance in utilising a scarce and expensive resource; and the transfusion thereof not risk free. To further this point Mirski and colleagues⁸ suggest that while a restrictive red blood cell transfusion strategy is beneficial in many clinical settings, the implementation in bleeding patients or those at high risk for haemorrhage may be hazardous. Furthermore, sustained anaemia in critical ill patients has been associated with worse outcome.⁹

Point of care testing, if found to be reliable, aids clinical judgment to determine the best patient management.

The close proximity of blood gas analysers in our theatre complex, the small amount of blood required and immediate availability of results add to the operational benefits.^{1,8,9,10,11}

Although a single ABG analysis in our setting costs approximately R40.00 versus a

laboratory based Hb of R15.62, the overall cost is reduced because the staff taking the sample also process it.¹² The cost of a single HemoCue cuvette to our institution is R10.34.

1.2 Aims and objectives

The hypothesis of this study was that POCT Hb concentration measurements by both the Ilex GEM PremierTM 3500 blood gas analyser (Instrumentation Laboratory, Lexington, MA, United states of America [USA]) and the HemoCue[®] Hb 201⁺ System (Mallinckrodt Medical, Hennef, Germany) would not differ from that determined by the central laboratory's, flow cytometry-based, Siemens Advia[®] 2120 system (Siemens Healthcare Diagnostics, Eschborn, Germany), i.e., the reference method.

The primary outcome was the between-device mean differences in Hb concentration measurements.^{13,14,15} A clinically relevant difference was considered to be a greater than 10% difference between the reference method and point of care device (POCD) measurements. The secondary outcome was whether, in the critical decision making range, the use of different devices would make a difference regarding the decision to transfuse or not, and was evaluated using an error grid analysis. This critical decision making Hb range is the Hb concentrations between 6 and 10 g/dL, where the decision is made on whether a transfusion is administered or not.^{16,17,18}

2. LITERATURE REVIEW

Point of care tests are seen as simple medical tests that can be performed at the bedside. However, such simplicity has not been achievable until technology had been developed, not only to make a test possible at all, but also to mask its complexity. As this technology infiltrates all sectors of clinical medicine, quality control, operator and device management, and compliance reporting are only a few of the challenges being faced beyond the generation of results.¹⁹

According to the International Council for Standardisation in Haematology (ICSH), the cyanmethaemoglobin assay (HiCN) is considered the gold standard or reference method for measurement of Hb levels in the blood.²⁰ However, a review of the literature by Berkow²¹ in 2013 showed that even standard laboratory measurements are subject to numerous methodological factors that affect both accuracy (how close the measurement is to the actual Hb value) and precision (how repeatable the measurement is). Bland and Altman²² also showed that both reference and test devices are subject to error and that these sources of error must be taken into consideration when assessing the accuracy of methodologies.

The ICSH defines a laboratory error as any defect, from ordering tests to reporting results and appropriately interpreting and reacting to these. The reported total laboratory error rates, which include pre-analytic, analytic, and post-analytic stages of testing, vary between 0.1 and 9.3% for laboratory measurements across the board.²³

Laboratory error is not the only potential source of variability in reported Hb values, numerous physiologic factors can also affect Hb measurement. Not only does arterial blood have a higher Hb concentration than venous or capillary blood, but the Hb level also tends to be higher with the use of a tourniquet for longer than 30 minutes, standing body position, blood sample taken in the morning, and can even differ between the left and right hand.²³

2.1 Studies pertaining to HemoCue®

The HemoCue is a portable Hb meter that uses the dry chemistry principle and has been widely used to check Hb levels in a variety of clinical settings. An overview of the literature provides evidence that the HemoCue is accurate and reliable, more so with venous or arterial blood than with capillary blood samples (especially in oedematous patients.) With finger prick samples (capillary blood) Bond and Richards-Kortum found that Hb concentration

measurement can vary by more than 2 g/dL in the span of two successive drops of capillary blood. They suggest that averaging the results of the droplet tests could produce values that were on par with venous or arterial blood tests, but tests on six to nine drops of blood were needed to achieve consistent results.²⁴

- In a recent systematic review and meta-analysis Hiscock et al.²⁵ assessed agreement between Hb measurement by HemoCue[®] photometers (201⁺ or B-Hemoglobin) and laboratory-based determinations, and concluded that the former was accurate. In the HemoCue group the overall mean difference was 0.08 g/dL (standard deviation [SD] 0.64 g/dL; 95% prediction interval -0.04 to 0.20) and 95% limits of agreement (LOA) -1.3 to 1.4 g/dL. The HemoCue[®] 201⁺ did not exhibit bias.
- Giraud et al.²⁶ investigated the accuracy of Hb assessment using bedside methods. The accuracy of the HemoCue device (arterial blood sample) was compared with the laboratory haematology analyser as the reference value. They studied 219 measurements from 53 adult patients undergoing surgery with expected blood loss. Haemoglobin laboratory concentrations values ranged from 6.8 to 16.3 g/dL. Compared with the reference method, bias was 0.2 g/dL for the HemoCue arterial sample (95% LOA -0.25 to 0.55 g/dL). This was true for paediatric surgery patients as well as adult aortic surgery patients. They concluded that none of the tested devices would have led to unnecessary or delayed transfusion according to the 2006 Transfusion Criteria of the American Society of Anesthesiologists.
- Seguin et al.²⁷ as well as Mimosz et al.²⁸ compared HemoCue values to a reference value in patients in the intensive care unit and reported similar bias, but wider precision of 0.5 and 1.0 g/dL respectively. They considered errors in blood sample handling as a possible explanation for the difference in findings. Seguin also recommended that capillary blood should not be analysed with a HemoCue in critically ill patients as tissue oedema greatly confounds results.
- Miller et al.²⁹ came to the same conclusion, namely that Hb assessment by HemoCue was consistently accurate with only one out of 78 HemoCue values measuring more than 1 g/dL from the reference value.

- In a study evaluating point of care Hb measurements using the HemoCue device for diagnosing anaemia, Schapkaitz et al.³⁰ compared the performance of the HemoCue with the Coulter[®] LH 750 automated haematology analyser. Accuracy, precision and linearity in 100 adult and paediatric patient samples were investigated. Samples from venepuncture, heel prick or arterial lines with volumes of more than 0.2 mL were used. The mean Hb value of the HemoCue (11.3 g/dL; range 4.6 to 16.7 g/dL) was comparable to that of the Coulter[®] LH 750 (11.3 g/dL; range 4.7 to 17.2 g/dL). The Bland-Altman difference plot revealed a good agreement. Bias between the two methods was small, and the imprecision was within acceptable limits. The investigators concluded that the HemoCue point of care device can be used in all age groups to provide accurate and reliable Hb measurements using smaller sample volumes, improved turn-around time, and long-term cost saving.
- Kim et al.³¹ studied different methods of determining the Hb levels of prospective blood donors, comparing Hb measurements from a non-invasive Hb sensor, the HemoCue (capillary sample) and an automated haematology analyser (venous sample) as the reference value. Their results showed that the average Hb values obtained by the HemoCue haemoglobinometer were 0.3 g/dL higher than the actual Hb value.

Previous studies appear to indicate that the HemoCue is accurate and reliable within 1 g/dL of the reference value over a wide spectrum of Hb values, provided capillary samples are avoided and good technique is practised.

2.2 Studies pertaining to blood gas analysers

While arterial blood gas measurement was traditionally implemented to measure acid-base status together with arterial oxygen and carbon dioxide tension, modern automated analysers also provide the clinician with a wealth of additional information – including the Hb concentration.

- Khanna et al.³² compared the intra-operative Hb measurements of 30 patients undergoing intracranial surgery. Haemoglobin values were measured by an arterial blood gas (ABG) analyser in the operating theatre and compared with an Hb estimation from the laboratory. Although this was not the primary outcome of their study, they reported a correlation coefficient of 0.97 between the ABG analyser and laboratory

values. Unfortunately, according to Carstensen et al.³³ using the correlation coefficient in this context is misleading, so these results should be viewed with caution.

- Rivas³⁴ investigated the comparison between a blood gas analyser and a Coulter counter for the measurement of Hb in 935 arterial samples collected during 314 liver transplants. Haemoglobin values obtained with the blood gas analyser (9.47 ± 1.74 g/dL) were, on average, higher than those obtained in the laboratory (9.17 ± 1.7 g/dL). In 8.1% of the samples, the blood gas analyser overestimated the laboratory value by more than 1 g/dL, but only in 0.3% of cases did it underestimate the laboratory value by more than 1 g/dL. The average difference between the two types of equipment was 0.30 ± 0.56 g/dL (95% CI 0.27 to 0.34 g/dL). The 95% CI for the lower limit of agreement was -0.88 to -0.76 g/dL and for the upper limit of agreement 1.36 to 1.49 g/dL (mean ± 2 SD).

In Rivas' study, 216 pairs of samples (23.1%) were collected in patients with Hb levels from 4.1 to 8.0 g/dL. No correlation was found between the differences in the two methods and the respective values of Hb ($r^2 = 0.0003$, $p = 0.64$).

The study included a significant number of paediatric cases and unexpectedly found that the differences between the two types of equipment were smaller and virtually negligible in the paediatric cases. A possible explanation is the special care that is taken with drawing blood from children and that the discrepancies were related more to the collection, handling, and transport of the samples than factors exclusively related to the equipment.

- Ray et al.³⁵ published similar results to Rivas³⁴, concluding that in critically ill adults, the ABG analyser provides a reasonable estimate of the Hb concentration over a broad array of values, but typically overestimates the Hb value by approximately 0.43 g/dL.
- On the other hand, a recent study of 348 patients undergoing multi-level spinal fusions by Carabini et al.³⁶ found that Hb values obtained from an ABG analyser and central laboratory complete blood counts (CBC) cannot be used interchangeably when managing a patient with critical blood loss.

The median difference (ABG-CBC) between the measured Hb values was 0.4 g/dL (95% CI 0.35 to 0.40 g/dL; $p < 0.0001$). Limits of agreement analysis correcting for

repeated observations in multiple patients demonstrated that the mean difference between measured Hb values (i.e., bias) was 0.4 g/dL (95% CI 0.36 to 0.41 g/dL), and the 95% LOA of the difference between paired measurements were -0.70 to 1.47 g/dL. The magnitude of the difference between the measured Hb values was >0.5 g/dL in 44.5% of the patients (95% CI 42.2 to 46.8%), however, 6.8% (95% CI 5.8 to 8.1%) of paired measurements had a difference of >1.0 g/dL. There was only a fair-to-moderate agreement between the CBC and ABG analyser values within the clinically significant range of Hb values of 7 to 10 g/dL (Cohen κ = 0.39; 95% CI 0.33 to 0.45).

Overall, the literature suggests that there is agreement between the laboratory Hb values and those measured by point of care devices. While the majority of measurements are within 1 g/dL of each other, there is however a regular breach of the 1 g/dL threshold. This is more commonly an overestimation (6-8% of values), but also occasionally an underestimation. The HemoCue may be slightly more accurate than the ABG analysers. Meticulous technique in acquiring samples seems to be a very important confounder.

3. METHODS

3.1 Research design

We conducted a prospective method comparison study of Hb measurement bias and accuracy in 58 adult cardiothoracic surgery patients between February and May 2014, at the Tygerberg Tertiary Hospital Complex, Cape Town, South Africa. The two cardiothoracic theatres run elective lists four days a week and offer a twenty-four hour emergency service.

Inclusion criteria were all male and female patients aged ≥ 18 years who presented for surgery at the Tygerberg Hospital theatre complex and who required arterial lines for clinical or surgical reasons. Patients with plasma sodium concentrations outside the range of 120 to 150 mmol/L were excluded as deranged values potentially cause inaccurate blood gas analyser Hb measurements.

Demographic information, relevant medical history, surgical procedures performed and vital signs were recorded. Information was initially recorded on paper in theatre, after which the data were captured on Excel spread sheets. Every patient was assigned a research number to protect anonymity.

3.2 Research instruments

3.2.1 Collecting of specimens

To prepare the wet heparinised sample 0.5 mL of 5000 μ /mL heparin (Heparin Sodium, Fresenius Kabi, USA) was drawn up into a 2 mL syringe to coat the walls of the syringe with the heparin. The excess heparin was then squirted out of the syringe. Gehring et al.⁴ has shown that using dried heparinised cuvettes versus wet heparinised sampling tubes can markedly reduce measurement errors. However, this holds true substantially more for the handheld systems than it does for the blood gas analysers. Dry heparinised cuvettes, however, are not readily available in our theatre complex and day-to-day blood gas analyses are done with wet heparinised syringes. To obtain results relevant to our clinical practise, we used the wet heparinised syringes for the blood gas analysers.

Arterial specimens were taken pre-, intra- or directly postoperatively. Three times the volume (9 mL) of the sample line was withdrawn from the arterial line to prevent haemodilution or contamination of the blood sample. This blood was injected back into the patient via a

peripheral or central line using a surgically clean technique, after the sample for analysis had been withdrawn.

The 3 mL blood sample was distributed between the wet heparinised sampling tube (1 mL) to be used in the two ABG analysers, 2 mL into an ethylenediaminetetraacetic acid (EDTA) BD Vacutainer[®] tube (BD Diagnostics, Franklin Lakes, NJ, USA) to be sent to the central laboratory, and a drop for the HemoCue[®] 201⁺ Microcuvette. These measuring microcuvettes served as a pipette and a reaction chamber. All the cuvettes were stored at room temperature and used within the expiry date and within 3 months of breaking the seal of the original storage container.

The wet heparinised samples were used for two identical Ilex GEM Premier[™] 3500 blood gas analysers, and the samples were analysed immediately.

The EDTA tube was sent at room temperature (25°C ±2) to the on-site National Health Laboratory Service and analysed using a Siemens Advia[®] 2102. The mean time from collection of the specimen to analysis was 6.2 hours (59% of the specimens were analysed within 6 hours). No specimens were analysed after 24 hours, Oddo et al.³⁷ having shown that stability of haematological analyses using the Siemens Advia[®] 2102 is not affected by EDTA room temperature storage for up to 24 hours.

To ensure consistent results, only the attending anaesthetists or qualified clinical technologists obtained the blood specimens and operated the POCT devices. The laboratory and clinical technologists were masked with respect to each other's results. All results were available to the attending anaesthetist for clinical decision-making.

3.2.2 Methods of haemoglobin measurement

3.2.2.1 Siemens Advia[®] 2120

The Siemens Advia[®] 2120^{b,5} flow cytometry-based system uses a cyanide-free method to measure Hb colorimetrically. After lysis of red blood cells, the heme ion is oxidised to the ferric state, which then combines with one hydroxide ion and one water molecule to form the axial ligand mono-aqua-mono-hydroxyferri-porphyrin. The optical readings are taken at a wavelength of 456 nm and plotted. According to Siemens the performance specifications and precision of the Advia 2120 for Hb measurements are 0.0 to 22.5 g/dL with a mean of 15.0 g/dL, SD of 0.14 and coefficient of variation of 0.93%.

The testing equipment was calibrated and tested as per the relevant laboratory standard operating procedures with control and calibrator products supplied by the manufacturer. The reference Hb measurements were performed using the National Committee for Clinical Laboratory Standards (NCCLS) H-15A approved standard method. The reference microhaematocrits were performed using the NCCLS H-7A approved standard method with tripotassium EDTA as the anticoagulant.³⁸

3.2.2.2 HemoCue[®] Hb 201⁺ System

The device uses a modified azidemethaemoglobin reaction for Hb quantification. Erythrocytes are haemolysed to release Hb, which is then converted to methaemoglobin and combined with azide. Absorbance of the azidemethaemoglobin is measured at dual wavelengths (570 and 880 nm) to compensate for turbidity.^{c,4}

According to Bäck et al.³⁹ the HemoCue[®] 201⁺ System compared well with other cell counters in their multi-site evaluation, producing a regression line of $y = 1.014x + 0.05$ with an average bias of 0.23 g/dL. Coefficient of variation for the HemoCue[®] 201⁺ System were 0.57 to 0.92% over the Hb range of 4 to 20 g/dL. (Reference article was supplied by the manufacturer).

It is to be noted that Gehring⁴ recommended the use of dry heparinised syringes as well as using the mean value of multiple samples for more accurate results.

The HemoCue[®] 201⁺ System devices are factory calibrated and need no further calibration.

3.2.2.3 Ilex GEM Premier[™] 3500 blood gas analyser

The GEM Premier[™] 3000 and 3500 blood gas analysers^{d,e,14} measure the haematocrit (Hct) using a technology called conductivity, and then calculates the Hb ($Hb = 0.31 \times Hct$).

Displayed ranges are as follows:

- Haematocrit: 15 to 65% with resolution of 1%
- Haemoglobin: 2.0 to 22 g/dL

Patel et al.¹⁵ reported the following values for the GEM 3000 in a study comparing measured Hb values with a reference analyser:

- Mean Hb: 8.67 g/dL
- Standard error: 0.1849739 g/dL
- Standard deviation: 1.5254334 g/dL
- 95% CI: 8.299908 to 9.038327

The intelligent quality management (iQM) of the Ilex GEM Premier™ 3500 runs after every blood gas analysis and every 12 hours. Via internal process control solutions and calibration validation solutions iQM provides continuous quality management and assessment of functionality with real-time error detection and correction and assures accurate results.⁴⁰ It is assumed that all the iQM cartridges used are similar.

3.3 Statistical methods

Our null hypothesis stated that there would be no statistically significant difference between the three POC devices and the laboratory measurement of Hb. The primary outcome was the between-device difference in Hb concentration measurements. A clinically relevant difference was considered to be a greater than 10% difference between the reference and POCD measurements (translating to a 1 g/dL difference in Hb at a total Hb concentration of 10 g/dL). The secondary outcome was whether, in the critical decision making range, the use of different devices would make a difference in the decision to transfuse or not. This range was to be evaluated using an error grid analysis, the critical decision making range for blood transfusion being between 6 and 10 g/dL.^{16,17,18}

A sample size calculation was done using statistical software (NCSS PASS, Release April 2007, Kaysville, Utah, USA; www.ncss.com). For a paired t-test where the null hypothesis is 10.0 and the alternative hypothesis is 9.0 with a known SD of 2.5 and alpha value 0.05, a sample size of 50 achieves 80% power. It was decided to study 60 blood samples in order to allow for missing values.

Haemoglobin concentrations measured by the four devices were compared, using analysis of variance for repeated measures.

Post hoc pairwise comparisons were done with the Bonferroni correction for multiple comparisons.

Bias and precision of the three POCD measurements were compared graphically with the standard laboratory measurements using the techniques of Bland and Altman.^{21,41}

Error grid analysis for paired Hb values was performed using the technique described by Clarke et al.⁴²

The statistical software used was MedCalc Statistical Software Version 13.1.2 (MedCalc Software bvba, Ostend, Belgium).

3.4 Limitations

As discussed in detail in the text, some confounding factors include the use of wet heparinised syringes and our relatively small sample size.

3.5 Ethics

Ethics approval was obtained (Protocol number S13/10/206) from the Health Research Ethics Committee of the University of Stellenbosch. This study was conducted in compliance with the submitted protocol, the International Council for Harmonisation, Good Clinical Practice guidelines and the applicable regulatory requirement(s). Formal informed consent was waived on the grounds that the research design involved no more than minimal risk and the tests performed were part of routine care for patients undergoing major surgery. All patients included in the study required arterial catheterisation for clinical or surgical reasons.

4. FINDINGS AND ANALYSIS

4.1 Demographic and baseline characteristics

In total, 58 patients, 35 males and 23 females, with a mean age of 50.8 years (range 24 to 82 years) were included in the study. Of the patients, four had received packed red blood cells (1 patient 1 unit, 2 patients 3 units, and 1 patient 4 units) before the study blood specimens were taken. None of the patients presented with active bleeding.

The most frequently reported medical history (Table 4.1) was hypertension in 29 patients (50.0%), hypercholesterolaemia in 16 patients (27.6%), and 15 patients (25.9%) had a smoking history.

Table 4.1 Medical history reported by >2% of the patients

Medical history (N=58)	n (%)
Hypertension	29 (50.0%)
Hypercholesterolaemia	16 (27.6%)
Smoker	15 (25.9%)
Diabetes mellitus	9 (15.5%)
Retroviral disease	8 (13.8%)
Rheumatic heart disease	5 (8.6%)
Stab chest	5 (8.6%)
Pulmonary tuberculosis	4 (6.9%)
Myasthenia gravis	2 (3.4%)
Subacute bacterial endocarditis	2 (3.4%)
N: Sample size; n: number of patients with the event	

The surgical procedure (Table 4.2) most frequently performed was coronary artery bypass surgery in 29 patients (50.0%).

Table 4.2 Surgical procedures performed on >2% of the patients

Surgical procedures (N=58)	n (%)
Coronary artery bypass graft	29 (50.0%)
Aorta valve replacement	10 (17.2%)
Mitral valve replacement	8 (13.8%)
Thoracotomy	6 (10.3%)
Thymectomy	2 (3.4%)
N: Sample size; n: number of patients with the surgical procedure	

4.2 Laboratory results

As up to two sets of samples could have been taken from a patient during the surgical procedure, there were 70 central laboratory Hb concentration (Laboratory haemoglobin) measurements and 58, 72, and 71 measurements for the HemoCue, blood gas analyser A (Blood gas A) and blood gas analyser B (Blood gas B), respectively. Of these, 14 of the measurements were obtained during cardiopulmonary bypass. Complete comparisons were available for 57 measurements; i.e., instances where values obtained from all three tested devices were compared with the central laboratory measurements. In 13 of the cases these measurements were repeat samples; but there were no more than two sets of samples drawn from any given patient. As such, correction for repeated measures from subjects was not considered to be significant and was not undertaken. Haemoglobin concentrations were in the clinically significant range of 6 to 10 g/dL in 54% (31/57), 44% (31/70) and 47% (33/69) of HemoCue, Blood gas A, and Blood gas B samples, respectively.

Repeated measures analysis of variance (Table 4.3) revealed that the mean Hb concentrations measured by all three POCD's were statistically significantly less than the central laboratory measurements ($p < 0.0001$) by 0.79 g/dL, 0.81 g/dL, and 0.67 g/dL for the HemoCue, Blood gas A, and Blood gas B samples, respectively. This represents an average underestimation of the Hb concentration by 7.2%, 7.4%, and 6.0% for the HemoCue, Blood gas A, and Blood gas B samples, respectively. The mean values of the three POCT devices did not differ statistically significantly from each other. The 95% CI of the differences between the mean values reveal that Blood gas A and Blood gas B both straddle the preselected clinically significant value of 1 g/dL, whereas the HemoCue's 95% CI of the difference did not reach clinical significance.

Table 4.3 Results of repeated measures analysis of variance on haemoglobin concentrations: Comparison of the three point of care devices with standard laboratory measurements

Device (g/dL)	Mean	SD	95% CI Diff
Laboratory	11.02	2.30	
HemoCue	10.23	2.23	0.47 to 0.87
Blood Gas A	10.21	2.49	0.43 to 1.15
Blood Gas B	10.35	2.52	0.46 to 1.16
CI: Confidence interval; SD: Standard deviation 95% CI Diff = 95% confidence interval of the difference between the laboratory mean value and the mean value obtained by the point of care device Laboratory = Siemens Advia® 2120; HemoCue = HemoCue® Hb 201 ⁺ System; Blood Gas A = Ilex GEM Premier™ 3500 blood gas analyser; Blood Gas B = Ilex GEM Premier™ 3500 blood gas analyser			

Results of the Bland and Altman analysis are presented in Table 4.4 and Figure 4.1, Figure 4.2, and Figure 4.3.

Table 4.4: Results of the Bland-Altman analysis: Comparison of the three point of care devices with standard laboratory measurements

	Blood gas A	Blood gas B	HemoCue
Sample size	70	69	57
Mean difference (g/dL)	0.69	0.66	0.67
SD	1.00	0.99	0.54
95% CI	0.44 to 0.93	0.42 to 0.90	0.52 to 0.81
p-value (H0: mean diff = 0)	<0.0001	<0.0001	<0.0001
Lower limit of agreement (g/dL)	-1.29	-1.27	-0.40
95% CI	-1.70 to -0.87	-1.68 to -0.87	-0.65 to -0.15
Upper limit of agreement (g/dL)	2.66	2.59	1.74
95% CI	2.25 to 3.07	2.19 to 3.00	1.49 to 1.99
Slope of regression line	-0.098	-0.013	-0.002
p-value	0.055	0.040	0.95
95% CI	-0.199 to 0.002	-0.201 to -0.005	-0.064 to 0.068
CI: Confidence interval; SD: Standard deviation 95% CI Diff = 95% confidence interval of the difference between the laboratory mean value and the mean value obtained by the point of care device Mean difference = average of (POCD measurements – Average (POCD measurements and laboratory measurements)) Lower and upper limits of agreement = mean difference \pm 1.96 SD Slope of regression line = slope of linear regression equation: Differences versus mean of (point of care device measurement and laboratory measurement) Laboratory = Siemens Advia [®] 2120; HemoCue = HemoCue [®] Hb 201 ⁺ System; Blood Gas A = Ilex GEM Premier [™] 3500 blood gas analyser; Blood Gas B = Ilex GEM Premier [™] 3500 blood gas analyser			

Figure 4.1: Bland-Altman analysis: Laboratory haemoglobin and HemoCue haemoglobin concentrations

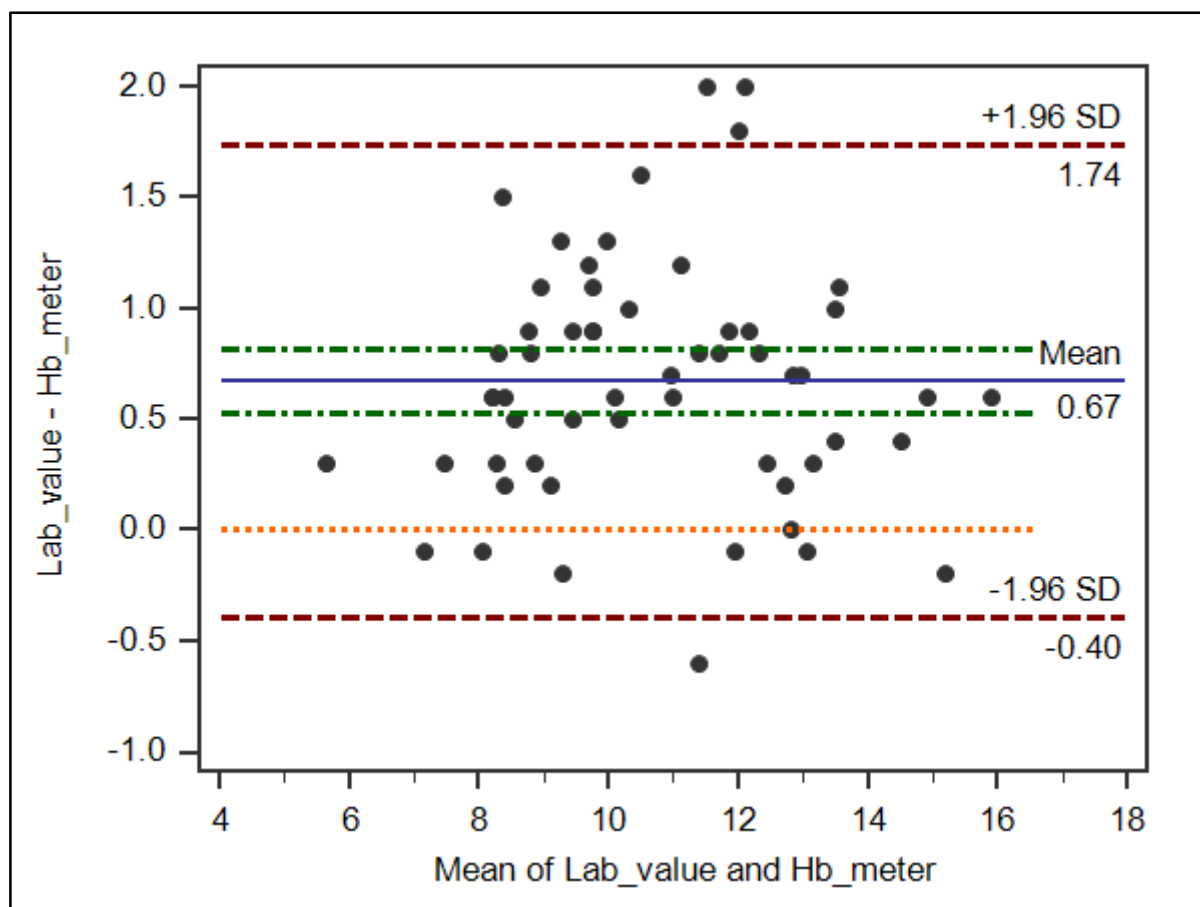


Figure 4.2: Bland-Altman analysis: Laboratory haemoglobin and Blood gas A haemoglobin concentrations

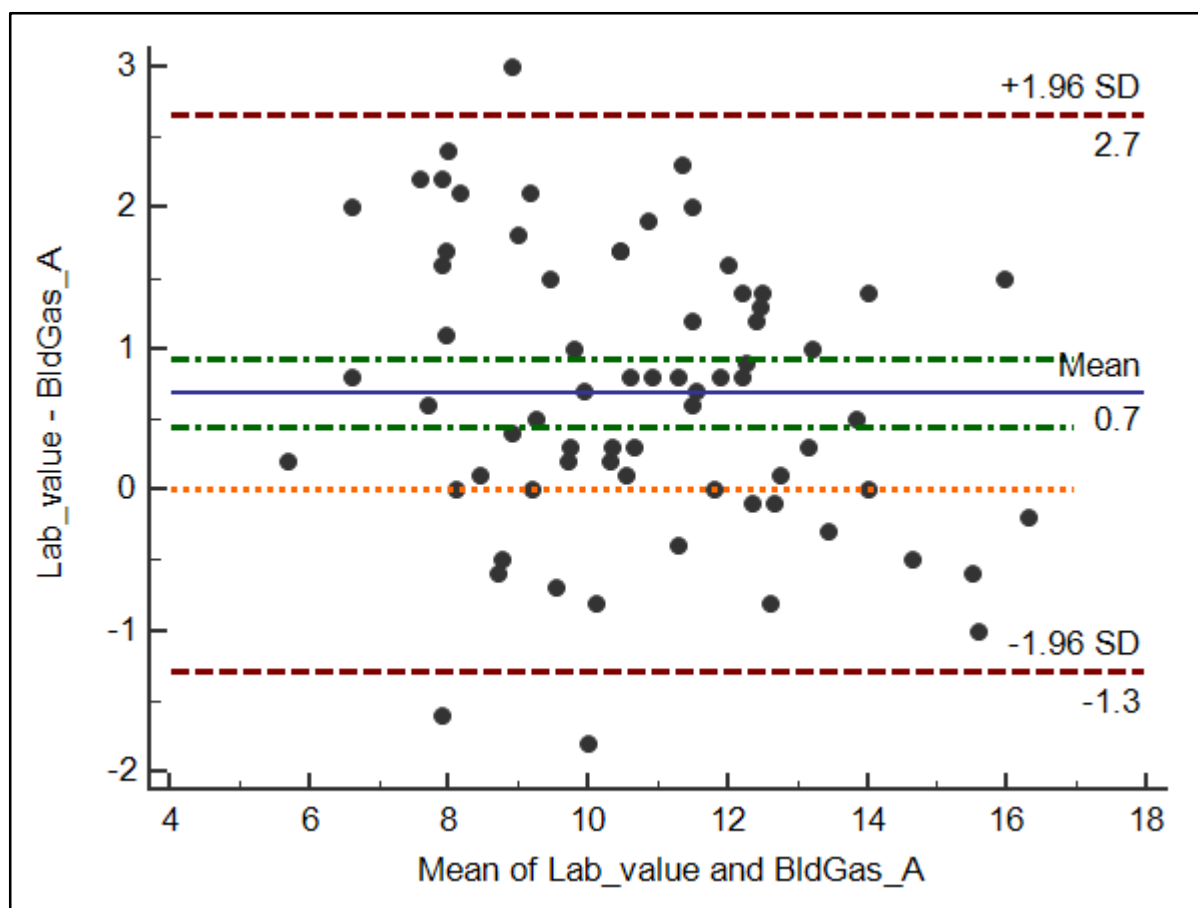
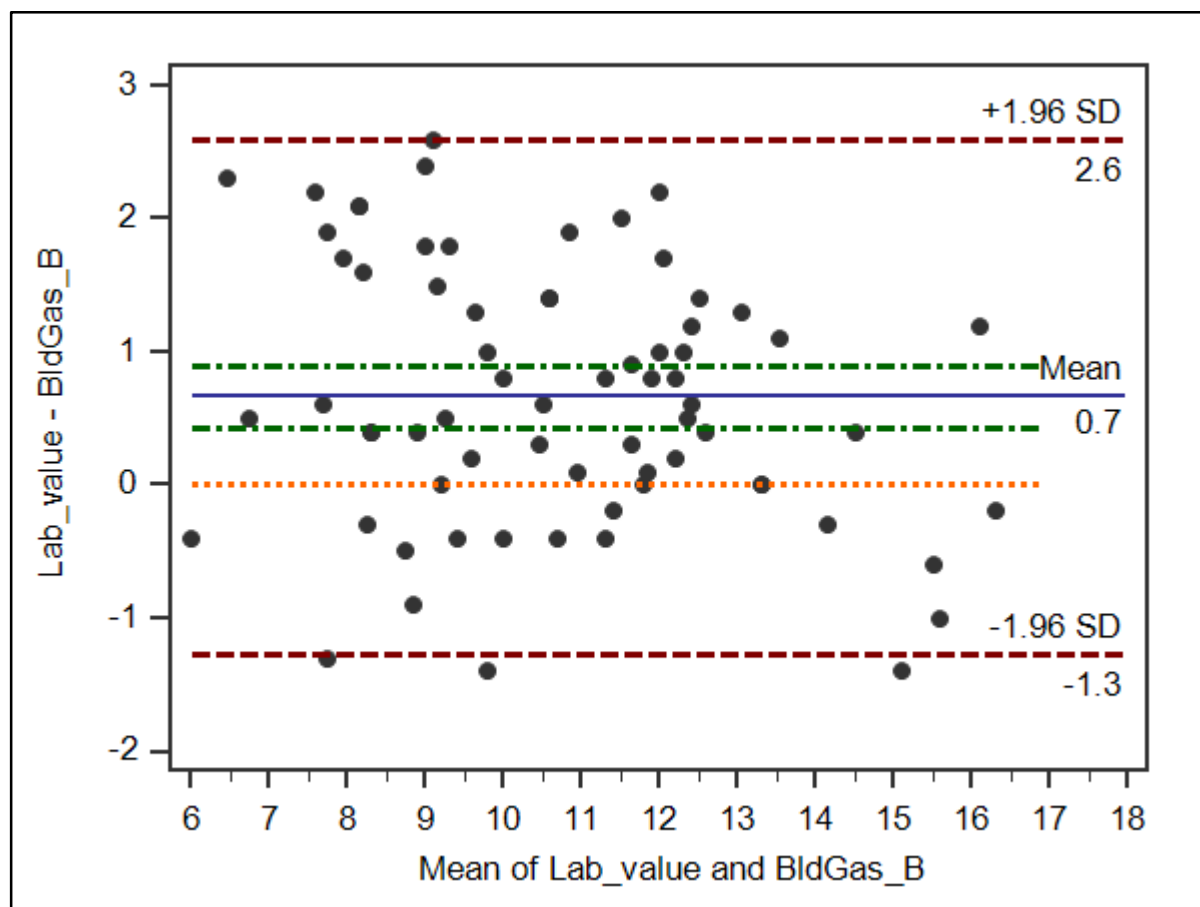


Figure 4.3: Bland-Altman analysis: Laboratory haemoglobin and Blood gas B haemoglobin concentrations



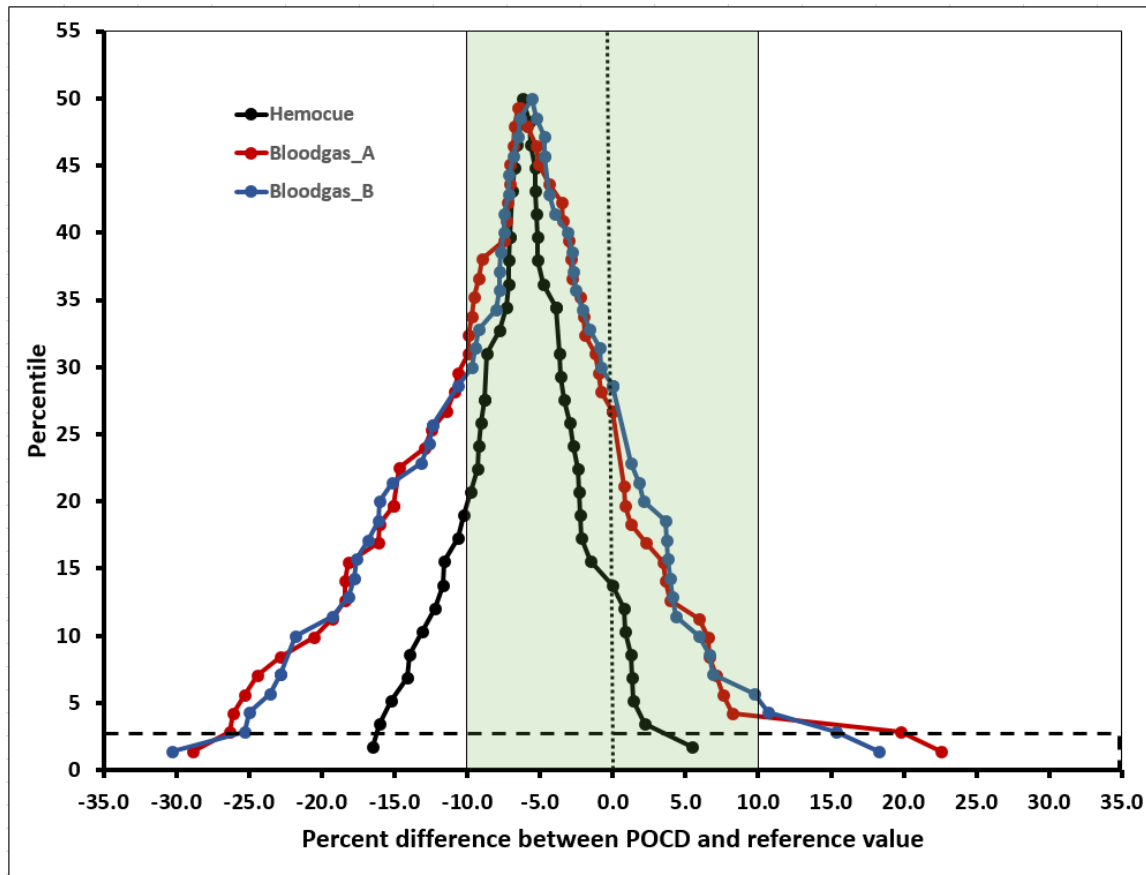
This analysis of the data showed that the POC devices had poor accuracy (bias) and underestimated central laboratory Hb values ($p < 0.0001$). Both blood gas machines underestimated the laboratory Hb values by 0.7 ± 1.0 g/dL (mean \pm SD), while the HemoCue only underestimated the central laboratory Hb values by 0.7 ± 0.5 g/dL. The 95% CI for the differences exclude zero in all three the POC devices indicating a statistically significant difference and that the Hb values obtained from the devices cannot be used interchangeably with the central laboratory Hb values.

Limits of agreement (LOA) for the difference shows a considerably larger spread for the Blood gas A (-1.3 to 2.7 g/dL) and Blood gas B (-1.3 to 2.6 g/dL) devices when compared with HemoCue (-0.4 to 1.7 g/dL). Furthermore, there is no overlap of the 95% CI of the LOAs (both upper and lower) of the Blood gas analysers and the HemoCue device. This proves a superior precision of Hb concentration analysis by the HemoCue device. As the

95% CI of the lower LOA for HemoCue (-0.65 to -0.15 g/dL) is less than 1 g/dL, the clinically relevant acceptable limit, determining the Hb with a HemoCue® Hb 201⁺ System device will not give overestimations, a possibility that exists for the blood gas devices' results.

The slope of the regression line was not different from zero for the HemoCue and Blood gas A, as the 95% CI of the slope includes zero. This demonstrates that there was no tendency for the differences to increase or decrease with increasing Hb concentrations (i.e., the differences were not proportional to the concentrations). For Blood gas B there was a small positive slope that was statistically significant, but not clinically important.

To complement the Bland-Altman analysis a folded empirical cumulative distributions plot, also known as the mountain plot, was constructed (Figure 4.4).^{43,44} This plots the rank (in percentile) of the size of the percentage difference (between the POCD and the laboratory result) against the percentage difference. The ranks are then folded around the 50th percentile rank. The apices of the plotted graphs confirm a similar median percentage difference, however the HemoCue demonstrates a narrower base, and less values outside of the shaded, clinically acceptable, 10% difference from the Laboratory result. This indicates better agreement and precision with the HemoCue device than with the Blood gas A and Blood gas B results.

Figure 4.4: Mountain Plot: HemoCue, Blood gas A and Blood gas B results

The secondary outcome of our study was whether, in the critical decision making range, the use of different devices would make a difference in the decision to transfuse or not. This critical range, being between 6 and 10 g/dL, was evaluated using a Clarke⁴² error grid analysis (Figure 4.5, Figure 4.6, and Figure 4.7). This indicated that 3/54 (5.6%), 14/66 (21.2%) and 12/62 (20%) values fell in Zone B for the HemoCue, Blood gas A, and Blood gas B POC devices, respectively. No values fell in Zone C.

Although the Clarke et al. glucose error grid includes five zones, we used three zones (A, B, and C) as suggested by Morey et al.¹⁶

- Zone A (Green)

As stated, a deviation of more than 10% of the tested technique from the reference method was considered clinically significant and formed a useful area in which one could expect 95% of all points to exist (consistent with the standard accepted type I

error). Only the HemoCue approximated this range with 94.4% of values in the Zone A versus 78.8% and 80% for the Blood gas A and Blood gas B devices, respectively.

In the uppermost field of Zone A (Hb concentration for both methods above 10 g/dL, with allowance for a 10% error), any bias in the two measurements will unlikely influence decisions concerning blood transfusion. This is because of a very high threshold for transfusion above an Hb level of 10 g/dL. In the lowermost field of Zone A (Hb concentration below 6 g/dL for both methods, with allowance for 10% error), the patient will likely be transfused irrespective of which method was used to measure the Hb. The more critical isthmus section of Zone A is the clinical decision making range (Hb concentration 6 to 10 g/dL) where the Hb may be a key determinant of whether a patient is transfused. In this most important section where reference and comparator device must most closely agree, the accuracy of the HemoCue in comparison with the blood gas analysers can be seen.

- Zone B (Yellow)

This area indicates significant errors in Hb measurement. Zone B is defined as the region between the upper line of Zone A and the upper Zone C and between the lower line of Zone A and the lower Zone C. Using the glucose error grid as an analogue,⁴² less than 5% of all points should be in Zone B. As stated above, 3/54 (5.6%), 14/66 (21.2%) and 12/62 (20%) fell in Zone B for the HemoCue, Blood gas A, and Blood gas B POC devices, respectively.

- Zone C (Red)

For this Zone, major therapeutic errors may occur. In the upper field upper Zone C, an Hb value measured by the new method would clearly overestimate the reference method. More importantly, an anaesthesiologist would likely not transfuse blood for a measured Hb of 10 g/dL, although the reference Hb may be 6.0 g/dL. In this case, failure to diagnose and treat anaemia may occur with potential injury to a patient. The lower Zone C region also predisposes towards major missteps in patient care in that a patient may be transfused unnecessarily. None of the tests performed on the POC devices fell into this Zone.

Figure 4.5: Clarke error grid analysis: HemoCue versus Laboratory haemoglobin

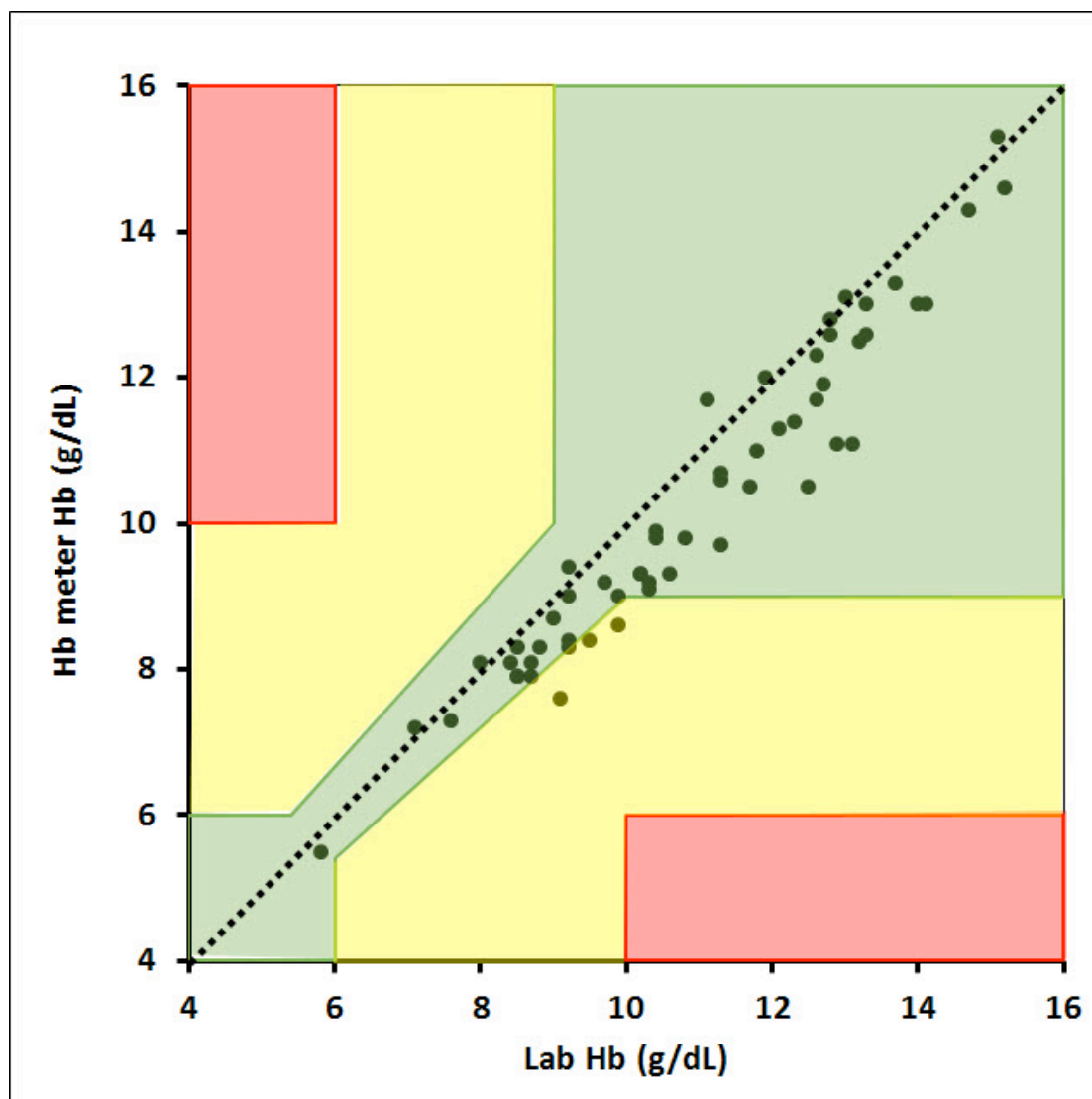


Figure 4.6: Clarke error grid analysis: Blood gas A versus Laboratory haemoglobin

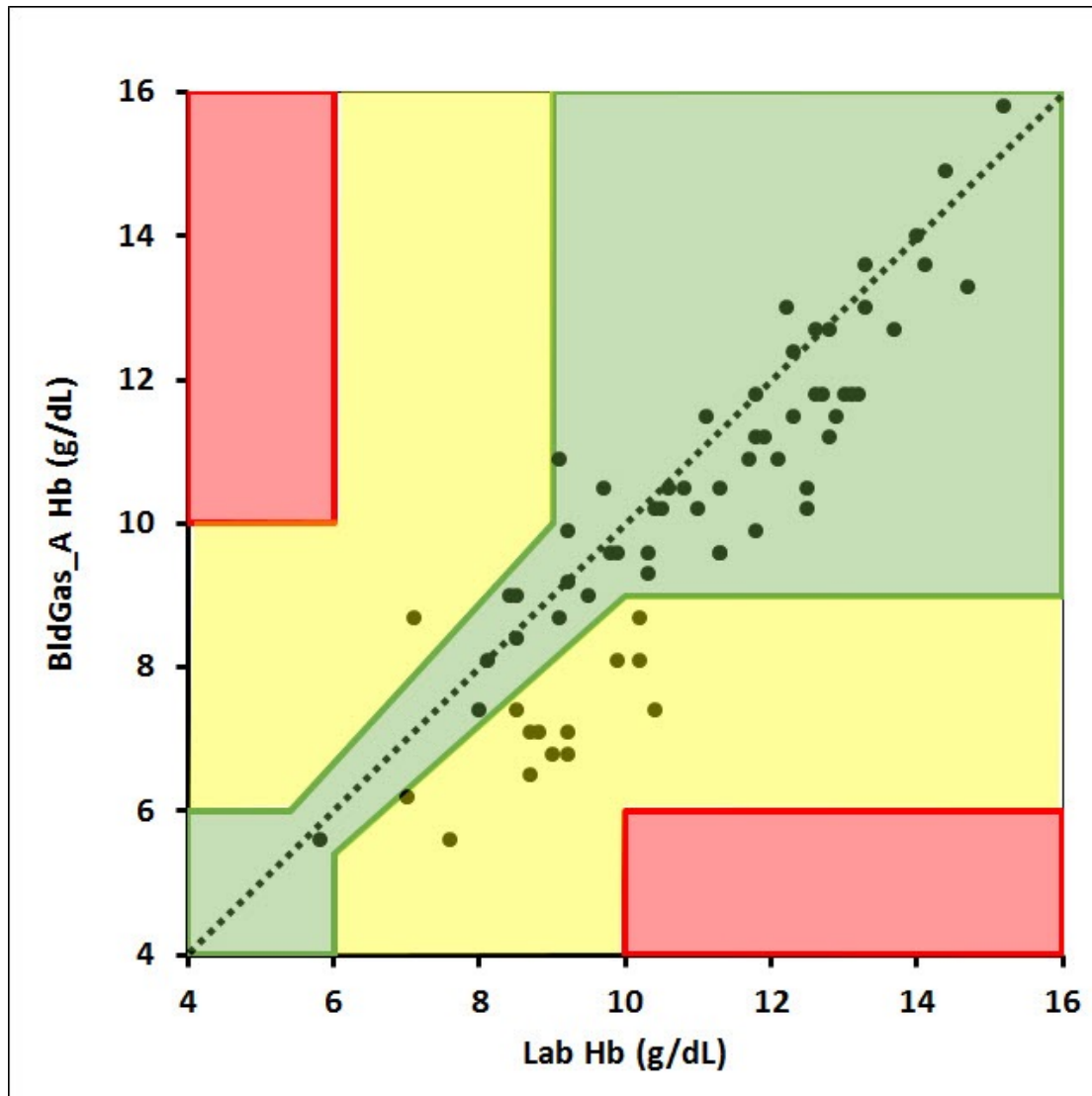
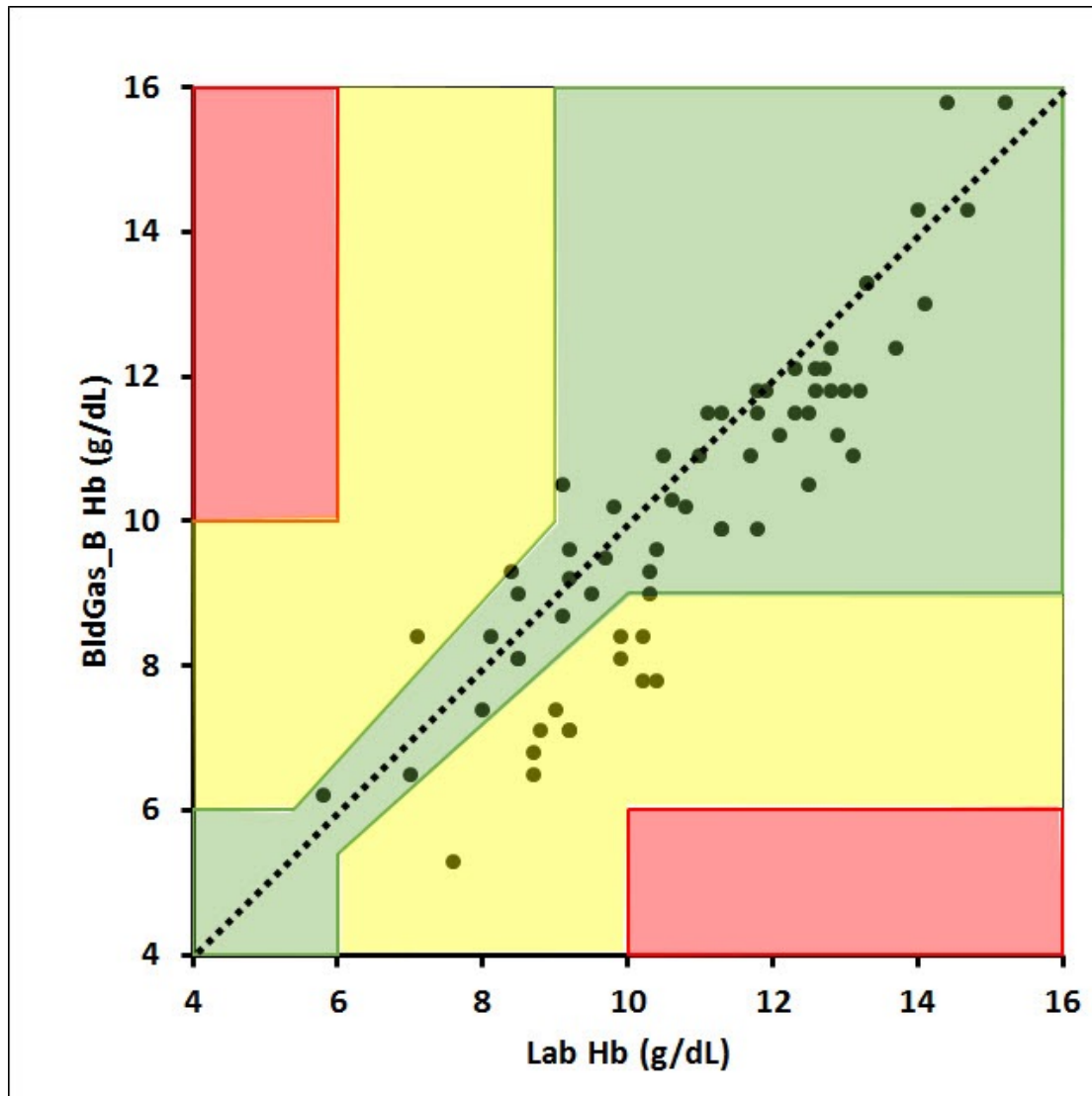
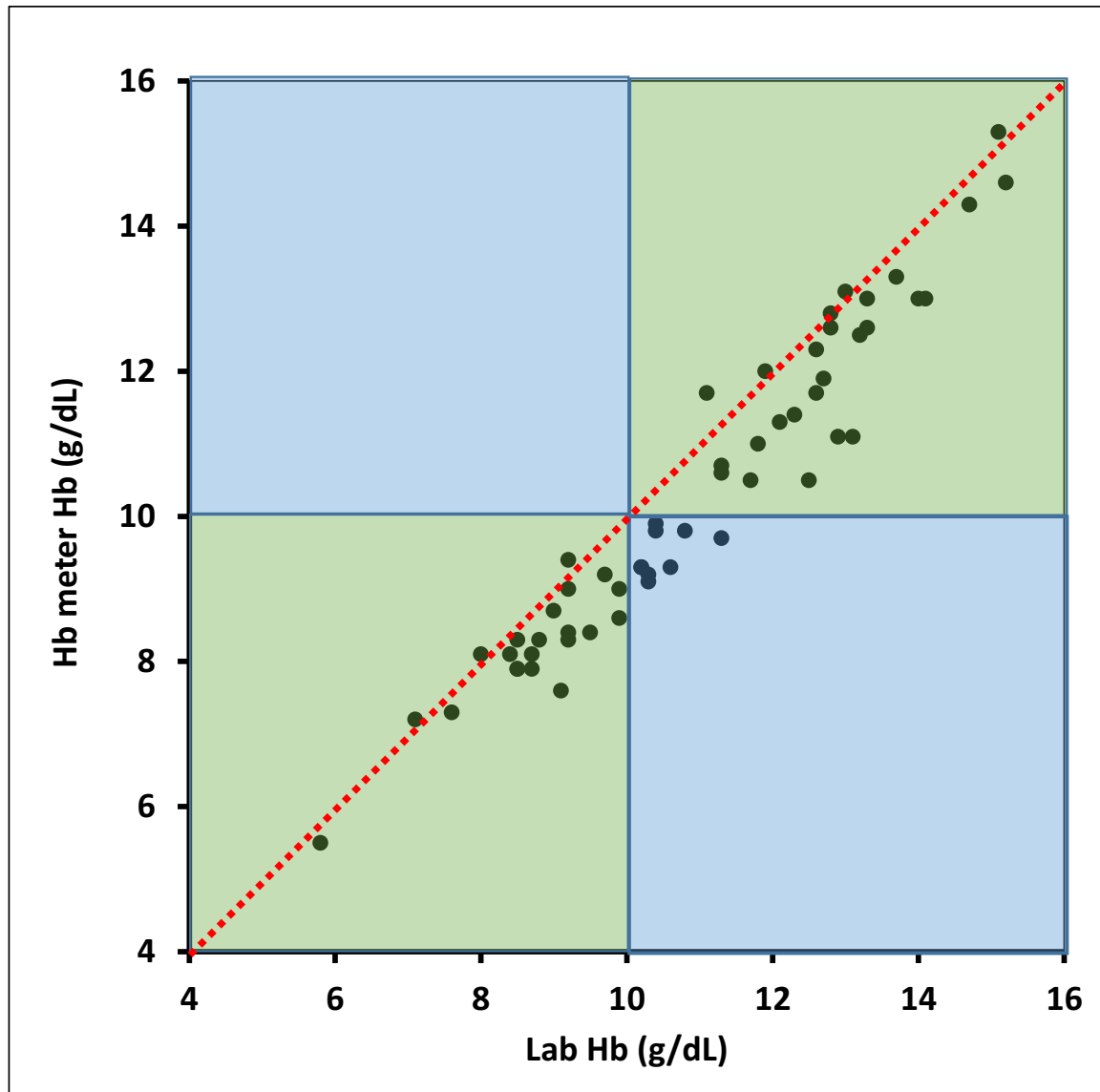


Figure 4.7: Clarke error grid analysis: Blood gas B versus Laboratory haemoglobin

It is important to note that the error grid is allowing for a 10% deviation of the POCD result from the laboratory result in the decision making range. A quadrant plot (Figure 4.8) illustrates the perils of making a transfusion decision based on the absolute value of the haemoglobin concentration derived from the POCD.

Figure 4.8: Quadrant graph of HemoCue haemoglobin concentration versus Laboratory haemoglobin concentration divided into quadrants by the 10 g/dL line of each method



In this instance the values that fall within the green quadrants represent the correct decision to transfuse a patient, in whom a 10 g/dL transfusion threshold cut-off was chosen. The values that are within the blue quadrants will lead to the incorrect transfusion decision, which in the case of this device (the HemoCue) will be the unintended administration of a blood transfusion. Similar analysis of the results from Blood gas A and Blood gas B confirms the aforementioned, and additionally introduce the possibility of unintentionally under transfusing patients.

A strength of our study is that approximately 50% of Hb values were in our defined clinical significant range of 6 to 10 g/dL. According to Morey and colleagues,^{16,17} recording a supermajority of Hb values >10 g/dL (values of minimal interest to the anaesthesiologist), when studying Hb measurement devices, biases the Bland-Altman analysis. If 90% of the data originate at Hb concentrations >10 g/dL then the mean value and SD become 90% weighted toward these values. This results in a device that could perform well in the upper range, but less accurately for values in the clinically more important range of 6 to 10 g/dL.^{41,42}

5. CONCLUSIONS

5.1 Summary of findings

We found that the mean POC (HemoCue, Blood gas A, and Blood gas B) Hb values underestimated the mean laboratory Hb by 0.79 g/dL, 0.81 g/dL, and 0.67 g/dL respectively. The difference was such that the results from these devices cannot be used interchangeably with those from the central Laboratory's analyser. The 95% CI of the between-method difference revealed that unlike the Blood gas A (0.43 to 1.15 g/dL) and Blood gas B (0.46 to 1.16 g/dL), the HemoCue (0.47 to 0.87 g/dL) values did not breach the 1 g/dL predetermined limit.

A Bland-Altman analysis revealed similar between-method mean Hb differences. However, the HemoCue upper and lower LOAs were narrower, without the 95% CIs of the LOAs overlapping those of Blood gas A and Blood gas B, indicating superior precision. Also, the 95% CI of the HemoCue lower LOA was less than 1 g/dL; this device does not give values that clinically overestimate the laboratory Hb.

There was no tendency for the differences between devices to increase/decrease over a wide range of Hb concentrations (i.e., the differences were not proportional to the concentrations).

An error grid analysis focused on the 6 to 10 g/dL Hb clinical decision-making range where only a 10% error was permitted. The error grid analysis revealed that the HemoCue[®] Hb 201⁺ System device and the two Ilex GEM Premier[™] 3500 blood gas analysers had potential transfusion errors of less than 5% and more than 20%, respectively. However, no devices produced values in the major therapeutic error zone.

5.2 Conclusion

The HemoCue[®] Hb 201⁺ System device performs with a superior clinical reliability and is less likely to result in transfusion errors than the blood gas analysers.

These findings are important in daily clinical practice and led to a change in the management of patients in the Tygerberg Tertiary Hospital theatre complex by staff now preferentially performing HemoCue Hb concentration measurements on venous or arterial samples if Hb estimation is the main endpoint. Repeating a sample and averaging the results may also improve accuracy.

5.3 Future research

Hypotheses for future investigations could be to explore the possibility that there is a difference in the size of the Hb measurement error on and off cardiopulmonary bypass and to investigate whether accuracy is improved by performing several measurements with a given device on the same sample.

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